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SEQUENTIAL SAMPLING PLANS FOR ESTIMATING GYPSY MOTH EGG MASS DENSITY

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The gypsy moth, Lymantria dispar (L.), is a serious defoliator of broadleaved forests in eastern North America. In addition, this pest defoliates trees and shrubs in urban/suburban areas. Defoliation of susceptible trees reduces tree growth and vigor. In combination with other stress factors (e.g. drought) and secondary organisms, defoliation can result in tree mortality.

The initiation of activities to suppress gypsy moth populations depends upon the results of biological surveys to estimate population density. These estimates are also used by managers to select the most appropriate gypsy moth suppression tactic (e.g. Bacillus thuringiensis, Dimilin®).

Historically, the egg stage of the gypsy moth has been surveyed as this stage is stationary and therefore easier to count. Viable eggs are



Figure 1.—Gypsy moth females and associated egg masses. (Photo by R.T. Zerillo)

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present for at least seven months (i.e., September through mid-April). Gypsy moth eggs are laid in late summer in a single egg mass per female that is covered by the abdominal hairs of the female (Figure 1).

This hairy covering, which has a buff-colored, velvety appearance when newly deposited, is visible (rubbing off the hairy covering exposes the individual shiny round eggs) (Figure 2). Females often deposit the egg mass in a slightly sheltered location: the underside of tree limbs, bark crevices, or under a rock overhang. In urban areas, eaves of houses, undersides of dog houses, bird houses, and other man-made objects are also used; vines that cover tree surfaces can hide many egg masses. Egg masses can be found in almost any location.



Figure 3.—Newly hatched first stage larvae on an egg mass.

Although the first stage larvae hatch from the eggs in the spring (Figure 3), the hatched eggs and egg masses themselves can persist. Thus, some of the egg masses in an area may be remnants from the previous year. Management programs are based on the newly deposited, viable egg masses. Newly laid masses are more firm, and the eggs are whole and opaque to dark-colored depending on the degree of development of the larvae inside. In contrast, eggs within last year's masses have exit holes and are more clear due to the lack of anything inside (Figure 4). The buff-colored abdominal hairs that cover the egg mass will, to varying degrees, lose their color with age (Figure 4).



Figure 4.—Newly laid and year old (faded color) egg masses. (Photo by R.T. Zerillo)

Egg mass size depends on the number of eggs in the mass. A healthy female generally deposits six to seven hundred eggs per mass, with some females depositing more than a thousand. These egg masses are relatively large, and are more common in low density populations. However, if a female has been stressed during larval growth, the number of eggs in a mass will usually be reduced to a few hundred or less, and these egg masses will be relatively small. It



Figure 2.—Individual gypsy moth eggs within the egg mass. (Photo by R.T. Zerillo)

is not uncommon to find mostly small egg masses in a population that is severely infected by the nucleopolyhedrosis virus (NPV) or in a population that has depleted its food reserves by defoliating the trees.

When designing a biological survey to estimate insect density, the precision and accuracy of this estimate depends partially upon: (1) the sampling technique used to collect the data and (2) the total number of samples used to make the estimate. A sampling technique is the method used to estimate density at a given location (e.g., searching the substrate). Accuracy and precision is increased by increasing the number of samples, but this must be balanced against the increased cost. Sampling plans are used to minimize the number of samples required to make correct management decisions, and thus minimize costs. In an effort to develop and evaluate sampling techniques and sampling plans for gypsy moth egg masses for use in an integrated pest management (IPM) program, the USDA Forest Service, Forest Health Protection, initiated cooperative work with the Northeastern Forest Experiment Station - Gypsy Moth Extramural Research Program, and scientists at VPI & SU and Penn State University.

SAMPLING TECHNIQUES

Some 13 different egg mass sampling protocols, mostly untested, have been developed among numerous agencies involved in gypsy moth management (Ravlin et al 1987). The three sampling techniques most commonly used to estimate gypsy moth egg mass densities are:

Fixed and Variable Radius (FVR) -- In the FVR technique (Wilson and Fontaine 1978), two substrates are searched: a 217.4 ft^2 (20.2 m^2) area on the ground, and trees chosen from the center of the area using a prism, such as a 20 basal area factor (BAF) prism. The number and location of trees searched depends upon their basal area and distance from the center of the circle.

Fixed 1/40th acre -- Fixed 1/40th acre samples (Kolodny-Hirsch 1986, Fleischer et al 1991, Liebhold 1991) are circles with a radius of 18.6 ft. (5.7m) - they are "fixed" because the radius

of the circle does not change (in the FVR technique, the area searched varies according to the BAF). The 1/40th acre area corresponds to 0.01 hectares. If the center of the bole of a tree falls within the circle, the entire tree is searched. In both fixed and FVR plots, trees are examined from all sides, and binoculars are used to search the higher portions of boles and crowns. Loose bark and rocks are overturned as the understory within the circle is searched.

Timed Walk -- Timed walks (Eggen and Abrahamson 1983, Fleischer et al 1991, Liebhold 1991) involve counting all egg masses seen while walking for a predetermined unit of time (typically five minutes). Alternatively, a predetermined distance can be used (Liebhold et al 1991). To gain precision, two persons conduct the walk simultaneously, and the two counts are averaged.

Comparison of techniques The FVR technique concentrates sampling on larger trees within a stand, and provides information on tree species composition. However, time constraints (0.46 to 1.0 person hour/sample, Wilson and Fontaine 1978, Kolodny-Hirsch 1986), limit their use in IPM programs. Timed walks vary excessively with respect to habitat and people conducting the work (Fleischer et al 1991, Liebhold et al 1991) - even one fixed plot per woodlot resulted in a more precise density estimate than multiple timed walks. IPM programs operate across a wide range of habitats, and timed walks and FVR plots were not designed to operate in habitats with a significant presence of man-made objects. Fixed 1/40th acre plots require less time than FVR plots, allowing for more samples, which becomes especially important when the data are used to make maps. Fixed plots also give the highest precision per unit time invested (Kolodny-Hirsch 1986). To provide precise density estimates across a wide range of habitats, fixed 1/40th acre plots were chosen as the sampling technique with which to develop sampling plans.

SEQUENTIAL SAMPLING PLANS

There is always a conflict between taking more samples to increase precision and the cost of taking these additional samples. A sampling plan balances these concerns. For gypsy moth management,

the primary concern is whether the egg mass density is above or below a critical threshold. Sequential sampling plans minimize sampling costs while maintaining accurate categorization of densities relative to a threshold. They accomplish this by concentrating sampling resources within geographic areas that are close to management thresholds.

Sequential sampling: theory -- Imagine a very large deck of cards, each with a number from 1 to 20 printed on it. Assume that the average of all the cards is 10. Also assume that there are lots of cards with the number ten (the average number), slightly fewer with the numbers 11 and 9, fewer still with the numbers 12 and 8, and very few with the numbers 2 and 18. If the number of cards for each value is plotted as a histogram (Figure 5), the histogram represents a frequency distribution - in this example the frequency distribution is a bell-shaped curve.

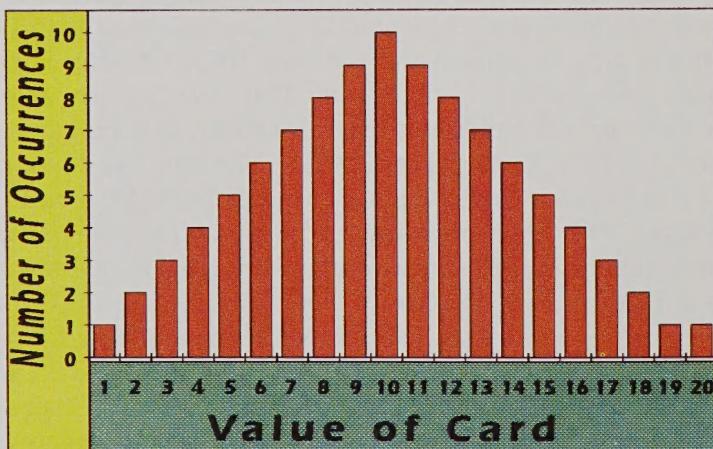


Figure 5.—Frequency distribution of cards in a deck.

You draw one card. There is a good chance it is the number 10 - there are more 10's than any other card. There is a smaller probability it is the number 2 - there are fewer 2's in the deck. You draw a second card. Again, it is more likely that it is the number 10, or 9 or 11. It is less likely that it is the number 2, and even more unlikely that you drew two 2's in a row. Thus, it is likely that your cumulative sum is close to 20, but unlikely that it is close to 4.

Sequential sampling plans use these probabilities (Figure 6). Let's say you have hundreds of decks of cards and your job is to find decks of cards in which the average is ten. In one deck you draw two cards, and the cumulative sum is four. You can say within certain levels of confidence that the average in this deck is less than ten, and a sequential sampling plan will tell you to stop sampling from that deck. In a second deck, the cumulative sum of two cards is 37. Again, you conclude that the true average is not ten, and you stop sampling. By stopping the sampling, the sequential sampling plan avoids more sampling than is needed to reach a decision. But in a third deck of cards, your first two samples add up to 18. The sampling plan tells you to continue sampling this deck. Labor is concentrated in decks of cards that are close to a threshold. In gypsy moth management, thresholds are average egg mass counts within a geographic area. The geographic area (e.g., a 1 kilometer square cell) is analogous to the card deck, and 1/40th acre plots analogous to the cards in the deck.

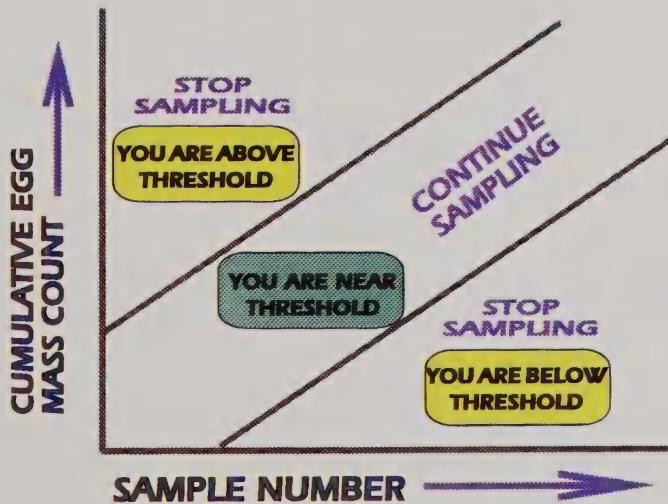


Figure 6.—Generic sequential sampling plan.

The sequential sampling plan estimates the probability of drawing the cumulative sum obtained from field samples if the true average equals a threshold. If that probability is low (the 1/40th acre plot counts are too low or too high), the plan recommends sampling be stopped. Otherwise, it recommends that another sample be taken, and the process of summing the field counts is repeated. This concentrates sampling resources into areas where the average is

close to the threshold, which increases the accuracy of the density estimate in those areas. Sequential sampling plans will reach a conclusion with as few as one sample, and will recommend sampling indefinitely when the true density equals the threshold. In practice, some minimum and maximum sample number is utilized, and the sequential plan used to adjust the amount of sampling effort between these extremes.

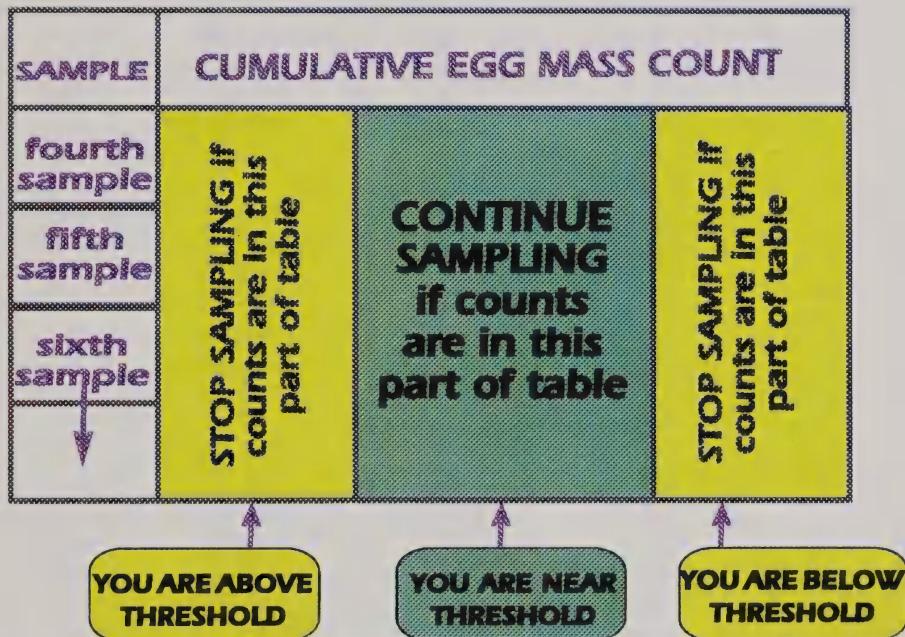
Two important assumptions were used: (1) a known threshold, and (2) a known frequency distribution (in the example it was a bell-shaped curve). The frequency distribution is used to estimate the probability of being above or below the threshold. A sequential sampling plan changes when these parameters change. A mathematical model is used to express the frequency distribution when the plan is designed and this model is built from previously collected samples. In forested areas, models describing frequency distributions were very similar between woodlots in Maryland (Kolodny-Hirsch 1986) and the mountains of Virginia (Fleischer et al 1991). Because gypsy moth egg masses are more clumped in areas with a lot of man-made objects, however, the models used to develop plans in urban/suburban areas are different from those in continuously forested habitats. Thus, plans are different for urban/suburban (areas with a significant amount of man-made objects present) versus forested habitats. And because more than one threshold exists in IPM programs, more than one plan exists for both habitats.

Sequential sampling in forested habitats -- Sequential sampling plans can be drafted as tables. To use these tables, first take the minimum number of samples required (in continuously forested habitat a minimum of four 1/40th acre egg mass samples is recommended). Distribute the samples to adequately represent the area of gypsy moth habitat (where eggs are likely to be found).

Sum the four samples. Compare that sum to the range of values in the "fourth sample" row of the table for the relevant threshold (Table 1). The plan will recommend to either stop or continue sampling. If the plan recommends to stop sampling, then move to the next area. Otherwise, take another sample, add it to your previous sum, and compare the new sum to the range of values in the "fifth sample" row. Continue until the plan recommends to stop sampling, or until you reach the maximum sample number, which is ten samples in

these tables. The tables only show the ranges for the fourth through ninth samples. At the ninth sample, you may be requested to continue sampling, so take the tenth sample. But ten is the last sample, regardless of the counts, so the row for the tenth sample was not printed.

Table 1.– Sequential Planning



Sequential Sampling Tables -- Gypsy moth management programs have different management thresholds in different parts of a project area. In the future, managers will be able to design and evaluate plans by interacting with a computer. Presently, separate sequential sampling plans exist for three management thresholds, and a separate portion of the table is provided for each plan (Table 2). Appendix 1 provides the equations used to generate the table.

Table 2.—Sequential sampling decision charts for three management thresholds in continuously forested eastern hardwoods.

Threshold (Egg masses/ acre)	Sample Number (1/40 acre)	Stop Sampling (below threshold)	Continue Sampling	Stop Sampling (above threshold)
.....Cumulative Egg Mass Count				
250	4	0-6	7-42	>42
	5	0-12	13-48	>48
	6	0-18	19-54	>54
	7	0-24	25-60	>60
	8	0-30	31-66	>66
	9	0-36	37-73	>73
500	4	0-15	16-81	>81
	5	0-27	28-94	>94
	6	0-39	40-106	>106
	7	0-52	53-118	>118
	8	0-64	65-130	>130
	9	0-76	77-143	>143
1000	4	0-18	19-178	>178
	5	0-43	44-202	>202
	6	0-67	68-227	>227
	7	0-92	93-252	>252
	8	0-116	117-276	>276
	9	0-141	142-301	>301

Sequential Sampling Plans for Urban/Suburban Habitats -- In areas that are influenced by man-made objects (urban/suburban habitats) the distribution of egg masses become more clumped than in forested habitats. Sample plans for urban/suburban habitats must reflect this difference in egg mass distribution. A sequential sample plan for urban/suburban habitats was developed in cooperation with Arlington, Fairfax and Loudoun Counties, Virginia. Urban/suburban sample plans were constructed using frequency distributions determined from 1/40th acre samples taken within one mile grid cells. The criteria for determining if an area was urban/suburban was based on housing density. For these sample plans, urban/

Table 3.—Sequential sampling decision charts for three management thresholds in urban/suburban habitats.

Threshold (Egg masses/ acre)	Sample Number (1/40 acre)	Stop Sampling (below threshold)	Continue Sampling	Stop Sampling (above threshold)
.....Cumulative Egg Mass Count				
250	6	0-3	4-71	>71
	7	0-9	10-77	>77
	8	0-15	16-83	>83
	9	0-21	22-89	>89
	10	0-27	28-95	>95
	11	0-33	34-101	>101
	12	0-39	40-107	>107
	13	0-45	46-113	>113
	14	0-51	52-119	>119
	15	0-57	58-125	>125
500	7	0-6	7-159	>159
	8	0-18	19-171	>171
	9	0-30	31-182	>182
	10	0-42	43-194	>194
	11	0-53	54-206	>206
	12	0-65	66-218	>218
	13	0-77	78-229	>229
	14	0-89	90-241	>241
	15	0-100	101-253	>253
	16	0-112	113-265	>265
	17	0-124	125-277	>277
	18	0-136	137-288	>288
	19	0-148	149-300	>300
	20	0-159	160-312	>312
	21	0-171	172-324	>324
	22	0-183	184-335	>335
1000	7	0-10	11-334	>334
	8	0-34	35-359	>359
	9	0-59	60-383	>383
	10	0-84	85-408	>408
	11	0-108	109-432	>432
	12	0-133	134-457	>457
	13	0-158	159-482	>482
	14	0-182	183-506	>506
	15	0-207	208-531	>531
	16	0-231	232-555	>555
	17	0-256	257-580	>580
	18	0-280	281-604	>604
	19	0-305	306-629	>629
	20	0-329	330-654	>654
	21	0-354	355-678	>678
	22	0-379	380-703	>703
	23	0-403	404-727	>727
	24	0-428	429-752	>752

suburban will be defined as areas with a minimum of one house per ten acres. Separate sample plans, each with a different set of minimum and maximum samples per square mile cell, are presented for three management thresholds (Table 3). Appendix 1 provides the equations used to generate the table.

Evaluation and Technology Transfer

Field evaluation involves comparing management decisions reached and amount of labor invested when the plan is used against the decision reached and labor invested when the maximum number of samples are taken. The plans described above for continuously forested habitat were evaluated in 132 one K² cells. The plans gave the same pest management decision in 79-84% of the areas which were surveyed by using the current operational techniques for the States of Maryland and Virginia. Only 2-3% of the areas resulted in incorrect decisions. The plans did not reach a conclusion, and thus recommended additional sampling, in the remainder. This rate of success was achieved with a savings of 40% of labor costs. Evaluation of the urban/suburban sequential sample plan was completed with field data from Loudoun and Arlington Counties, VA. The plans gave the same pest management decision in 90-100% of the 1 mile grid cells. The plans did not reach a conclusion in 3% of the cells and 6% were incorrectly classified. Use of the urban/suburban sample plans resulted in a 49% saving in labor cost. The plans developed in continuously forested habitats have now been used in the collection of over 80,000 samples across two years within the Appalachian Gypsy Moth Integrated Pest Management Program (AIPM). The urban sequential sample plans have recently been developed and evaluated, and have not been used during a field season.

Summary

Sequential sampling plans have been designed using fixed radius plots to help allocate labor when determining if gypsy moth densities exceed a threshold density for an area. These plans were originally developed in forested habitats, where they provide correct pest management decisions with a 40% reduction in labor costs. They have recently been expanded for use in habitats where a significant presence of man-made objects exist, such as urban/suburban habitats.

For additional information concerning the estimation of gypsy moth egg mass density, contact either Jane Carter - Blacksburg, VA 24061 at (703)231-4161, Dr. Shelby Fleischer - University Park, PA 16803 at (814)863-7788, Dr. Richard Reardon - Morgantown, WV at (304)285-1566, or your local Cooperative Extension Service office.

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Appendix 1.—Equations used to generate sequential sampling plans for 1/40th acre fixed plot samples in various habitats.

Threshold (EM/Acre)	Decision Stop Line ^a	
	Continuously Forested Habitat	Urban/Suburban Habitat ^b
250	$y = 6.095x \pm 17.722$	$y = 6.089x \pm 34.013$
500	$y = 12.178x \pm 32.706$	$y = 11.781x \pm 76.258$
1000	$y = 24.580x \pm 79.461$	$y = 24.576x \pm 162.128$

^aDecision stop line in the form $y = mx \pm b$, where y = cumulative sum required to stop sampling, m is the slope, x is the sample number and b is the intercept. The positive value of the intercept gives the upper stop line and the negative value gives the lower stop line.

^bDefined as ≥ 1 house per ten acres.

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